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NUTRITION REVIEWS

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NUTRITION REVIEWS

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EPIDEMIOLOGY AND NUTRITION SURVEYS

Workers in the field of communicable diseases have long recognized three basic approaches to knowledge of causation and behavior of disease, the ultimate objective being improved prevention and control and better clinical management. One approach is the study of the individual patient by clinical methods; another is through laboratory investigation. The third general method is epidemiologic, the study of population groups.

Epidemiologic studies were among the earliest in nutrition. Original concern was with spectacular epidemics of the time, which rivaled those of infectious origin to such extent as to engender a belief that they were indeed communicable processes. In much the same way that infectious agents so long dominated medical thought about communicable disease, the vitamins have had overemphasis in the death, defect, and disability associated with nutritional disorders.

The basic pattern by which the epidemiologic method is turned to practical use in communicable and other disease holds equally for nutritional disorders. It involves, in order, a definition of the nature and extent of the problem, and a search for factors of causation, both of the disease and of its distributions as seen in nature in populations of people. There follows a formulation of principles for a program of control, these principles being based on demonstrated cause and directed toward death, defect, and disability; and finally an evaluation by field and statistical methods of results obtained from control measures.

The remarkable gains in knowledge during recent years have been incentive enough for workers to center effort on the specific problems of nutrition, and so far as field studies are concerned, "most published results of nutrition surveys indicate that the purpose was to discover deficiencies of vitamins" (*National Research Council Bull.* **117** (*1949*)). An extension of field activities to broader epidemiologic interests is conceivably to the advantage of nutrition and to public health generally.

The term "nutrition survey," as used by nutritionists, is a generic expression for a variety of activities, some of which the epidemiologist would recognize as field surveys and some not. All involve collection of data in the field. Case finding is the simplest of all epidemiologic procedures and much used in nutrition. It involves no selection of specific groups for study. The field survey, to the epidemiologist, is examination of selected samples of a population within a prescribed and limited time, with the purpose of determining quantitatively the frequency of existing cases of disease. This is the short-term, cross-section prevalence study. It commonly involves comparison of observed results with similar situations in other population groups, or with an accepted normal value.

A somewhat different procedure also identifies affected members of a population sample, but has as its objective not the determination of existing total frequency, but estimation of the rate at which new cases appear within successive periods of time, commonly years. This is the long-term incidence study, and in combination with prevalence determination serves as an index of the dynamic behavior of a disease.

The technics of field observation have been used also for administrative purposes. Data on food supplies, food habits, food consumption, and health conditions of groups of persons have served in health education and for public enlightenment on means for improved social and economic conditions.

The recent bulletin of the National Re-

search Council (*loc. cit.*) presents American practice critically and in detail, together with an extensive bibliography on nutrition surveys. Successive sections are devoted to objectives of nutrition surveys, dietary surveys, chemical tests and their interpretation, clinical diagnosis in nutrition surveys, and the part that surveys have in community-nutrition programs, in the training of nutritionists, and in the work of health departments.

Epidemiologic study of disease in the field makes use of three principal scientific disciplines, those of clinical medicine, of the laboratory, and of biostatistics. This holds equally in application to nutritional disease. In nutrition surveys, as in other fields of medicine, the principal difficulty has been in defining a normal value (J. A. Ryle, *Lancet* **I,** *1* (*1947*)), whether that normal refers to an individual or, as in surveys, to a community. Determination of the range of normality is further complicated by lack of good correlation between clinical and biochemical observations (E. Riggs *et al.*, *Canad. J. Pub. Health* **34,** *193* (*1943*)). Some of the reasons for the observed irregularities have been discussed by H. M. Sinclair (*Am. J. Pub. Health* **34,** *828* (*1944*)), W. J. Darby (*J. Am. Dietet. Assn.* **23,** *204* (*1947*)), and P. Putnam *et al.* (*Milbank Memorial Fund Quart.* **27,** *355* (*1949*)).

The nutrition survey has had wide application to a variety of population groups. Increasing emphasis is being placed on the nutrition survey that gives attention to the general health of communities as well as to nutritional states (J. B. Youmans, E. W. Patton, and R. Kern, *Am. J. Pub. Health* **32,** *1371* (*1942*); **33,** *58* (*1943*); D. F. Milam and R. K. Anderson, *South. Med. J.* **37,** *597* (*1944*); Milam and W. J. Darby, *Ibid.* **38,** *117* (*1945*)). Surveys of diabetes, heart disease, and tuberculosis are profitably combined with nutrition surveys, with attention to the social, hygienic, and psychologic constitution of populations. It is important to assess the part of nutritional disorders as a direct influence on the health of communities; it is also essential to know the effect they have on other mass diseases.

As nutrition surveys commonly involve selected populations, the basis for selection requires clear statement, and the group studied is to be carefully defined. Putnam *et al.* (*loc. cit.*) have given attention to these matters as they relate to nutrition, and the British studies on hemoglobin (*Med. Res. Council, London, Special Report Series* **252** (*1945*)) are especially informative as to method.

A pilot survey to test efficiency and adequacy of methods is an essential part of any large survey. There is general need for a clearer description of pathologic differences within a particular nutritional entity, and for quantitation of their frequency in observed populations.

Some of the problems in determining what is a normal state of community nutrition conceivably could be solved by developing means for measurement that lie outside strictly nutritional characteristics; for instance, objective tests of function in relation to food intake. This would be a test of what a community could do on a particular dietary regimen (H. A. Kraut and E. A. Muller, *Science* **104,** *495* (*1946*)) rather than of the physical or biochemical abnormalities it shows. Various tests of physical performance (J. R. Gallagher and L. Brouha, *Yale J. Biol. Med.* **15,** *657* (*1943*)) are in the same category.

More universal use of technical refinements of the epidemiologic method should also contribute to results from nutrition surveys. The relation of rates of prevalence and of incidence is a common means of determining the dynamics of mass disease of populations. Nutritional field studies commonly compare rates of prevalence, determined at intervals in a particular community, but the combined indices are not so frequently used as in the communicable diseases.

Increasing reference is found in the lit-

erature of nutrition surveys to the need for an analyst. The greater need would appear to be a "synthesist." Too many surveys are organized on the premise of collecting all possible data pertaining to the situation, and then calling in a statistician to find out what has been discovered. The basic need is for a proper design of experiment before operations begin, first to state clearly the question to be answered, and secondly to develop a planned procedure to accomplish that objective. A more extended discussion of the role of epidemiology in nutrition is to be found in a publication by J. E. Gordon and H. le Riche (*Am. J. Med. Sci.* **219,** *321 (1950)*).

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LIPOTROPIC ACTION OF CHOLINE, BETAINE, AND METHIONINE IN BIRDS

The lipotropic factors (choline, betaine, and methionine) have received considerable attention in fatty-liver studies with rats, dogs, and mice, and to a limited extent with other species. Comparatively little is known concerning the role of these compounds in the lipid metabolism of birds. Shortly after Best and associates made the observation that choline prevented accumulation of excessive amounts of fat in the livers of rats, preliminary investigations with other species indicated that mice responded similarly, but inconclusive results were obtained with chickens and dogs (C. H. Best, M. E. Huntsman, and O. M. Solandt, *Tr. Roy. Soc. Canada* (*Section V*) **26,** *175 (1932)*). In the experiment with chickens the average fatty acid content of the livers of 4 control birds was 7.7 per cent and of 5 test birds, which received 200 mg. of choline per day, 3.3 per cent.

O. D. Abbott and C. U. DeMasters (*J. Nutrition* **19,** *47 (1940)*) obtained limited data which indicated that choline supplements to a semipurified diet lowered the liver-fatty acids in pullets.

D. M. Hegsted, R. C. Mills, C. A. Elvehjem, and E. B. Hart (*J. Biol. Chem.* **138,** *459 (1941)*) confirmed, with chicks, the earlier observation made by T. H. Jukes (*Ibid.* **134,** *789 (1940)*) with turkeys, that choline was essential for prevention of perosis and promotion of growth. They were, however, unable to demonstrate any lipotropic action of choline. The livers of the choline-deficient chicks appeared normal at autopsy, and analysis revealed 3.93 per cent fat (fresh tissue basis) compared with 3.89 per cent fat in the choline-supplemented group.

It is generally believed that the main function of choline in avian nutrition is that of preventing perosis and promoting growth, and that betaine and methionine can partially replace choline in these functions.

Some Canadian workers have reported that choline has a lipotropic function in the nutrition of ducklings in addition to being required for growth and for prevention of perosis (R. Bernard and J. M. Demers, *Canad. J. Res.* (*Section E*) **27,** *281 (1949)*). They further reported that neither methionine nor betaine could completely replace choline in its lipotropic action. A significant reduction in liver-fatty acids occurred when an 18 per cent casein diet, patterned after that of A. Roos, D. M. Hegsted, and F. J. Stare (*J. Nutrition* **32,** *473 (1946)*), was supplemented with 0.1 or 0.3 per cent choline. The livers of 6 ducklings receiving the basal diet had a 14.4 per cent lipid content, whereas the livers of a like number receiving either 0.1 or 0.3 per cent choline contained 8.0 per cent lipids. Supplementing the diet with 0.5 per cent betaine resulted in an average liver-lipid content of 10.8 per cent, a value which they did not consider statistically significant compared with the 14.4 per cent average of the controls. A diet of

commercial duck pellets gave liver-lipid values of 6.0 per cent in a comparable experimental period (three to four weeks).

A diet containing only 9 per cent casein was employed in order to determine lipotropism for methionine. No significance could be ascribed to the results since growth was very poor and liver lipids varied considerably within all groups. In an initial test, 0.5 per cent DL-methionine supplement increased the average liver lipids from 34.3 per cent (S.E. 7.48) to 47.7 (S.E. 2.39), but in a repeat experiment the liver lipids were nearly the same in both groups (29.7 and 29.8 per cent). In the latter experiment, a supplement of 0.3 per cent choline reduced liver lipids to 17.5 per cent and a combined supplement of 0.5 per cent DL-methionine and 0.3 per cent choline gave a value of 14.8 per cent. However, neither of these values was considered significant because of the wide variations observed in birds having the same treatment.

Data were obtained on still another diet which was designed to correct some of the apparent multiple amino acid deficiencies noted with the 9 per cent casein diet. It contained 15 per cent skim milk powder in addition to 9 per cent casein. With this diet neither choline nor methionine alone exhibited a significant effect, but a combination of them lowered liver lipids significantly. The liver-lipid values were as follows: no supplement, 16.7 per cent; 0.3 per cent choline chloride supplement, 13.2 per cent; 0.5 per cent DL-methionine supplement, 21.8 per cent; and 0.5 per cent DL-methionine with 0.3 per cent choline chloride supplement, 10.4 per cent. The rise in liver lipids noted here with methionine and a similar rise noted in the initial test with the 9 per cent casein diet (see above paragraph) led the authors to the assumption that methionine was antilipotropic. A statistical analysis, however, failed to show that this methionine effect was significant.

From the data it is apparent that nearly normal liver lipids were achieved only when choline was added to the 18 per cent casein diet. Unfortunately methionine was not tested with this diet. When a 9 per cent casein diet was used, together with 15 per cent skim milk powder, choline became significantly lipotropic only when combined with methionine. It would have been interesting, therefore, to have established the effectiveness of methionine on the 18 per cent casein diet.

The authors suggest that, on the basis of their results, marked differences exist between the chick and the duck with respect to liver-fat accumulation and liver-fat transport.

Roos, Hegsted, and Stare (*loc. cit.*) reported several years ago that the duckling is very susceptible to perosis on a choline-deficient diet. The Canadian workers observed perosis consistently in the ducklings which received the 18 per cent casein diet unsupplemented with choline. This condition did not develop in the slow growing ducklings receiving the 9 per cent casein diet and appeared in only 3 of the 6 ducklings receiving the diet of 9 per cent casein combined with 15 per cent skim milk powder. Their results suggest that the duck resembles the chick in that neither betaine nor methionine can completely replace choline for perosis prevention and growth promotion. Neither betaine nor methionine was tested in combination with low levels of choline. Since these substances can partially replace the choline requirement for the chicks when diets of similar type are used, it would be of interest to know whether this is true for the duck.

The report of a significant lipotropic action of choline in ducklings is of interest. It should serve to stimulate a more extensive examination of the significance of choline and other lipotropic factors in the lipid metabolism of growing chicks and especially of laying hens. The latter are subject to a high level of lipid metabolism in view of the high-fat

content of egg yolk and more particularly in view of the high level of choline-containing phospholipids in egg yolk.

In view of the vast amount of work already done on the effect of choline, betaine, and methionine on the promotion of growth and prevention of perosis, it is unusual that practically no reported data are available on the possible lipotropic function of these substances in the chick.

ENRICHMENT OF DIET WITH PROTEIN

Not only are the presently popular problems of geriatrics being examined with reference to nutrition, but the influence of diet upon structural and physiologic well-being during early adult life has been the subject of continued study. In a series of such careful evaluations, H. C. Sherman, C. S. Pearson, and M. E. Bal (*Proc. Nat. Acad. Sci.* **36,** *106 (1950)*) have made a quantitative investigation of the influence of protein enrichment of an adequate laboratory diet. The laboratory diet (No. 16) consisted of wheat and milk powder with a protein content of 14 per cent; for the experimental ration poultry meat was added to raise the protein content to 24 per cent. Beginning with a modest sample of 40 young female rats on each dietary regimen, it was found that 7.5 per cent of those receiving the laboratory diet died between the ages of 1 month and 1 year, whereas on the ration with enhanced protein content, 17.5 per cent died during the same period.

Other results of enrichment with protein are assessed: thus the data show a marked increase in body weight in the group consuming the meat-enriched diet, with high statistical significance. This stimulation to growth was accompanied by an earlier development of sexual maturity as shown by earlier reproductive performance. Another index of success in reproduction, namely, the number of young born and the number reared, per female, was essentially the same in both groups of rats. On the other hand, the body weights of the young at the end of infancy showed a significant difference in favor of those from mothers consuming the protein-enriched diet. The span of reproductive life of the mothers showed no difference which could be attributed to the difference in composition of the experimental rations.

As might be expected, the foregoing adjustment is not without demonstrable shifts in retention of other dietary essentials. Some twenty years ago, M. Kriss and A. H. Smith (*J. Nutrition* **16,** *385 (1938)*) demonstrated that severe restriction of inorganic salts markedly influenced the nature of the mixture of organic fuel materials being oxidized in the body, and later this was shown to be true when calcium alone was deficient (M. Kleiber, M. D. D. Boelter, and D. M. Greenberg, *Ibid.* **19,** *517 (1940)*). At about this time, H. C. Sherman, H. L. Campbell, and C. S. Lanford (*Proc. Nat. Acad. Sci.* **25,** *16 (1939)*) observed that on the wheat and milk ration, which has a relatively low-calcium content (0.18 to 0.20 per cent of the air-dry mixture), the calcium content of the body increased less rapidly than did the body weight; this resulted in a drop in the percentage of calcium in the carcass despite the support of a positive calcium balance during the period of observation. Furthermore, when increase in body weight was accentuated by adding protein to the wheat and milk diet, the tendency to diminished calcium storage progressed to the point where symptoms suggestive of actual calcium deficiency appeared (Sherman and C. S. Pearson, *Ibid.* **33,** *264, 312 (1947)*; **34,** *585 (1948)*; Sherman, M. S. Ragan, and M. E. Bal, *Ibid.* **33,** *356 (1947)*). While spontaneous recovery usually occurred, some of the females thus stimulated to rapid development and

early puberty, suffered disaster in reproduction. Further studies of the relation between protein and calcium in the diet will no doubt be forthcoming.

TOXICITY OF D-AMINO ACIDS

In common with many other organic compounds of biochemical importance, most of the amino acids arising from digesting protein exhibit steriochemical specificity. Obviously, this has been demonstrated most cogently with the so-called essential amino acids; thus only the L-form of lysine, threonine, valine, leucine, and isoleucine will satisfy the needs of rapid growth when suitably supplemented with other dietary essentials. However, the growth-promoting capacity of the D-modifications of phenylalanine, tryptophan, and methionine is nearly as great as that observed with the natural (L) forms, though D-histidine is less effective in this respect than the optical isomer (G. J. Cox and C. P. Berg, *J. Biol. Chem.* **107,** *497 (1934)*).

In addition to the biologic ineffectiveness of certain of the D-isomers, the question of their actual toxicity has been raised, to explain failure of growth when rations containing the DL-forms of several of the essential amino acids were fed (A. A. Albanese and V. Irby, *Science* **98,** *286 (1943)*).

Among others who have questioned the toxicity of the D-modifications of the indispensable amino aicds are J. F. Van Pilsum and C. P. Berg (*J. Biol. Chem.* **183,** *279 (1950)*). These investigators used mixtures of optical isomers of the essential amino acids in both high- (25 per cent) and low- (2 per cent) fat, but otherwise adequate, diets composed of purified substances. One amino acid mixture provided twice the minimal amounts (W. C. Rose, *Science* **86,** *298 (1937)*) of the essential amino acids in their L-form; the second mixture contained the same amount of the L-amino acids but supplied as the DL-modifications. The third mixture supplied half as much DL-arginine, tryptophan, phenylalanine, and methionine as the second mixture, an intermediate level of D-histidine, and the same amounts of the other five essential amino acids. Growth on the second ration was poorer than on the first ration, but when allowance was made for the growth-promoting properties of the D-forms of phenylalanine, methionine, tryptophan, and arginine by giving half as much of the DL-forms (third ration), growth was as good as when only the L-amino acids were present (first diet). In addition it was demonstrated that growth retardation with the second diet was due to the comparatively high level of DL-methionine; when this amino acid was diminished in concentration by 50 per cent, the retarding effect of the second ration was eliminated.

The foregoing results demonstrate that the D-forms of the essential amino acids are not toxic even when fed (as in the second diet) at an elevated level. Indeed, when L-methionine was substituted in the second diet for the DL-mixture, growth of the experimental animals was less favorable than before, indicating that where both isomers of an amino acid promote growth, the natural form may be more toxic than the D-modification as shown, in this instance, with methionine.

NITRATE IN VEGETABLES

Nitrates in animal feeds may be reduced by bacteria in the digestive tract, especially of ruminants, to nitrites. Nitrites in sufficient quantity may cause poisoning through reac-

tion with hemoglobin of the blood to form methemoglobin. The resulting removal of hemoglobin as an oxygen carrier is somewhat analogous to the action of carbon monoxide or hydrogen sulfide. The acute symptoms, in cattle, which result from nitrate poisoning have been referred to as "oat-hay poisoning."

In man, the effect of nitrate ingestion in food has not been well defined. H. H. Comly (*J. Am. Med. Assn.* **129,** *112* (*1945*)) has described cyanosis, in infants, that could be correlated with the nitrate content of well water used to make the formula for the babies. This cyanosis was due to methemoglobinemia and its development was attributed to reduction of the nitrate to nitrite in the gastrointestinal tract of the infant with diarrhea. In 1947, 33 cases of this syndrome in Illinois were ascribed to the presence of excessive nitrate in drinking water used to make infant formulas (*Science News Letter* **53,** *275* (*1948*)); 5 cases are said to have resulted in death. After this experience a maximum safety limit of 10 parts per million of nitrate nitrogen in water was set by the Illinois authorities. The use of these waters by adult members of the families does not appear to have produced disease.

Infant-feeding practices usually include the early introduction of vegetables into the diet. Vegetables contain nitrates, the extent of which is not always appreciated. Numerous fresh green vegetables grown at Ithaca, New York were analyzed for nitrate by J. K. Wilson (*Agron. J.* **41,** *20* (*1949*)). Beets, broccoli, cabbage, cauliflower, celery, and head lettuce contained over 1000 p.p.m. of nitrate. Carrots, cucumber, curly lettuce, and muskmelon contained less than 1000 p.p.m.; rhubarb contained as much as 5400 p.p.m. Considerable variation was found among samples, as would be expected of plants from different soils and cultural practices; only tomatoes were found to contain no nitrate.

Juices of green vegetables and quick frozen foods were also studied. Samples of spinach, rhubarb, and celery were high in nitrates and several other vegetables were frequently high also. Among strained baby foods, spinach and beets were especially high, whereas tomatoes, peas, and squash had no nitrate. The juice from a sample of fresh-frozen spinach contained 6600 p.p.m., or the equivalent of 7 per cent potassium nitrate on the dry basis.

Thus it is apparent that certain foods may contain nitrate. Wilson (*loc. cit.*) expresses unjustified concern for the effect on human health of the quantities of nitrate in vegetables and speculates that the "high quantity of nitrate . . . may be attributed . . . to the application of nitrogenous fertilizers." In some species of plants the content of nitrate is related to the amount available to the growing plant. This effect has not been defined for vegetables. Although heavy nitrate fertilization is a common commercial practice to produce vigorous, bright green leafy and succulent vegetables, there is no evidence that such fertilization results in accumulation of quantities of nitrate which have proved toxic for man. Indeed, other than the relation cited above between nitrate in well water and methemoglobinemia in infants with diarrhea, evidence is lacking for the toxicity to man of nitrate in food.

In herbivora, however, high-nitrate content of feed is associated with cornstalk disease, oat-hay poisoning, borna disease of horses, and grass tetany of cattle. Wilson's paper indicates the need to determine whether the conditions in the human gastrointestinal tract, like those in the alimentary canal of herbivora, may favor nitrite production from nitrate. Also, there is need for reliable information on the variation of nitrate content of foods with changes in nitrate treatment of soils. However, on the basis of existing evidence there seems to be no cause for the concern expressed by Wilson for the possibility of widespread disease in man due to nitrates in plants as a result of the use of fertilizers containing nitrates.

DESIRABLE FAT LEVELS IN THE DIET

The optimum amount of fat in the diet has not yet been established. The Recommended Dietary Allowances of the National Research Council place the desirable fat level in the diet at 20 to 25 per cent of the total caloric intake. For very active adults, children, and adolescents the desirable level is placed at 30 to 35 per cent of the total calories. This subject was reviewed in 1947 (*Nutrition Reviews* **5,** *258 (1947)*). Evidence which supports the view that the type of fat fed may have a bearing on the quantity needed in the diet for optimum growth in the rat, has been presented by V. H. Barki, R. A. Collins, C. A. Elvehjem, and E. B. Hart, workers at the University of Wisconsin (*J. Nutrition* **40,** *383 (1950)*).

With butterfat as the dietary fat, greater gains resulted as its concentration in the diet was increased. Three levels of butterfat were tested, namely, 10, 28, and 35 per cent. In each of three experiments involving a total of 20 weanling rats the highest level of butterfat produced the greatest weight gains. The difference in gains between 10 and 35 per cent butterfat in the diets assumed statistical significance in two of the three experiments reported. The average weight gains for all three experiments were 187.4, 204.5, and 220.3 g. with the 10, 28, and 35 per cent butterfat diets, respectively, for the six-week experimental period.

Parallel studies were carried out with corn oil as the dietary fat constituent. Statistically significant differences in gains with varying levels of corn oil were observed in one of the two experiments reported, involving a total of 14 rats. In contrast to the results observed with butterfat, corn oil produced the best gains when it was present in the diet at a level of 10 per cent, although there was very little difference between the 10 per cent level and the 28 per cent level (208.9 g. for 14 rats receiving the 10 per cent corn oil diet and 206.6 g. for 20 rats receiving the 28 per cent corn oil diet).

The gain in 20 rats receiving the 35 per cent corn oil diet was 196.4 g. compared with the previously mentioned value of 220.3 g. observed in the 20 animals receiving the 35 per cent butterfat diet.

The diets used in these studies were so compounded that the ratio of calories to proteins, salts, and vitamins remained constant with increasing levels of fat. Casein was the protein source and was included at a level of 0.04615 g. per calorie of diet. Sucrose served as the dietary carbohydrate component. In the experiments described above, the rats were allowed to eat as much as they wanted.

In another experiment in which the same levels of corn oil and butterfat were compared, the animals were pair-fed, that is, they were restricted in food consumption to the least amount consumed by any single rat in the study. During the entire experiment the lowest food consumption consistently occurred among the animals receiving the diet containing 35 per cent corn oil. There was again a significant increase in gain with increased dietary butterfat content. Six-week gains were 169.4, 173.8, and 179.8 g. with 10, 28, and 35 per cent butterfat, respectively. Gains over a comparable period with corn oil diets were 174.5, 182.1, and 176.7 g. with 10, 28, and 35 per cent corn oil, respectively. The gains on the diet containing 28 per cent corn oil (182.1 g.) were significantly greater than those obtained with the same level of butterfat (173.8 g.).

No correlation was evident between corn oil level in the diet and efficiency of energy utilization (grams gain per 100 calories absorbed). Increased levels of dietary butterfat consistently improved the efficiency of utilization of absorbed energy.

In both the liberally-fed and the pair-fed studies the coefficient of digestibility of corn oil was superior to that of butterfat. Values for corn oil ranged from 96.1 to 97.3, whereas

values for butterfat ranged from 90.8 in pair-fed rats receiving the 10 per cent butterfat diet to 93.7 in either liberally-fed or pair-fed rats receiving the 28 per cent butterfat diet.

A deficiency of essential fatty acids was considered by these investigators as a possible cause for the reduced growth on low levels of dietary butterfat. A source of one of the essential fatty acids, ethyl linolate, however, failed to improve such diets. These workers had earlier observed that orally administered commercial liver extract (reticulogen) counteracted the growth-depressing effect of diets high in corn oil. In the present study, reticulogen failed to improve the 28 or 35 per cent corn oil diets when it was administered by intraperitoneal injection.

Diets containing 10, 28, or 35 per cent soybean oil or coconut oil were also tested. Changes in the dietary level of these fats did not alter the growth significantly.

The results may be summarized as follows: Rats consistently gained more weight when fed a diet containing 35 per cent butterfat than when fed a similar diet containing corn oil. In contrast, when the diets contained only 10 per cent of these fats, growth was consistently better on the corn oil. The intermediate level of 28 per cent corn oil or butterfat in the diet produced about equal weight gains under liberal-feeding conditions, but corn oil produced significantly better gains when the animals were pair-fed. The poorer weight gains on the high-corn oil diet could not be corrected with liver extract and ethyl linolate did not improve the low-butterfat diet.

Earlier reports have indicated that the type of fat may influence the dietary level required for optimum growth. Thus R. Hoagland and G. G. Snider (*J. Nutrition* **22,** *65 (1941)*) observed that steam-rendered lard and hydrogenated cottonseed oil had the same growth-promoting value when the diet contained 5 per cent fat, but lard was superior when the diets contained larger proportions of fat.

H. J. Deuel *et al.* (*J. Nutrition* **33,** *569 (1947)*) observed that optimum growth in male rats was obtained when the diet contained 30 to 40 per cent margarine, whereas the optimum level for cottonseed oil was 20 per cent.

H. J. Deuel and co-workers (*J. Nutrition* **40,** *351 (1950)*) have also tried to determine whether fat contributes to nutrition other than as a source of essential fatty acids or as a reservoir for meeting caloric needs.

The growth of rats receiving a low-fat diet and a source of essential fatty acids (60 mg. of methyl linolate daily), was accelerated by including 10 per cent cottonseed oil in the diet. The possibility that the fat addition might have improved the palatability of the diet and thus increased food consumption was not precluded. Records of food consumption were not reported.

The improved growth noted with the addition of cottonseed oil to the diet could also be explained on the basis of the results of E. B. Forbes and associates (*Nutrition Reviews* **3,** *82 (1945)*; **5,** *100 (1947)*). These workers have demonstrated conclusively that fat exerts a beneficial influence by improving the efficiency of utilization of the carbohydrate and protein with which it is fed.

The impaired growth noted with low-butterfat diets in the experiments of the Wisconsin investigators might be attributed to a lowered efficiency of utilization of calories consumed. They observed decreased efficiency of energy utilization with decreasing dietary butterfat levels. The impaired growth with high levels of corn oil, however, cannot be explained on this basis. No correlation was evident between the dietary level of corn oil and the efficiency of utilization of calories.

Evidence is thus accumulating that, in the rat at least, fats may have varying influences in nutrition and that the amounts needed in the diet for optimum growth may depend, to a considerable extent, upon the source of the fat.

TREATMENT OF MENTAL DEFECTIVES WITH THIAMINE

Aneurine, the British term for thiamine, suggested in 1935 by the Dutchman B. C. P. Jansen, points to the antineuritic effects of the vitamin in peripheral neuritis of nutritional origin. Its effects on the cerebral processes are less well defined. Several recent studies on thiamine intake and intelligence were reviewed in this journal (*Nutrition Reviews* **7,** *220 (1949)*).

The possibilities of using thiamine for the treatment of mental defectives were investigated by G. de M. Rudolf (*J. Ment. Sci.* **95,** *910 (1949)*), visiting psychiatrist at the Hortham Colony, England. The total number of individuals of all grades of mental deficiency—idiots, imbeciles, feebleminded—in the colony was 687. Out of these, a series of 93 certified mental defectives of both sexes, all ages (between 5 and 55), and all grades of deficiency, who showed no improvement—and, presumably, no deterioration—for at least a year prior to commencement of the vitamin therapy, were chosen as subjects. These patients had been in the colony for periods varying from three to seventeen years. They were given orally 3 mg. of thiamine once daily for five to six months. The 3 mg. daily dose was considered as the advisable maximum. Even with this dose, which would be generally considered low, increased excitability ascribed to the increased thiamine intake was observed in 5 subjects. The increase in 3 patients was severe enough to necessitate termination of treatment within three months. The thiamine content of the diet was not determined and no control group of subjects was used.

Mental deficiency has an organic basis, but the concept of mental deficiency is defined in social terms, behavior and social adjustment being of paramount importance in judging fitness for normal life. In the present study three social criteria were considered: the general behavior in the lodges, the performance at school or at work, and the social age as measured by Doll's social maturity scale. In addition, the subject's intelligence quotient was determined before and after treatment. Three intelligence tests were applied, depending on the chronologic age and the mental level of the patient, but for any one individual the same test was used on the two occasions.

The results were discussed in reference to the four criteria described above. The behavior in lodges remained stationary in 73 patients, 12 showed improvement (in 5 cases the improvement was considered slight), and 8 showed deterioration. The latter figure includes the 3 cases in which the treatment had to be discontinued after three months. These 3 patients will not be considered in the discussion to follow.

Twenty-five patients had intelligence too low to attend school or to do any kind of work. Of the remaining 65, 52 patients remained stationary, 10 showed improvement (which was termed slight in 5 cases), and 3 deteriorated in school or at work.

Taking the whole group of 90 patients, the social age remained stationary in 36 cases, rose in 36 cases, and decreased in 18 cases. The mean social ages, calculated from the data given in the paper, were 3.60 years before treatment and 3.78 years after the treatment. The social ages were determined on two occasions preceding thiamine treatment; considering only the 37 patients in which the same social-age estimates were obtained, the criterion remained stationary in 14 cases, increased in 16, and decreased in 7 cases.

The average intelligence quotient of all 87 patients for whom the records were available was 32.7 before and 30.4 after the treatment. These values were calculated from Table V in the original article. The intelligence quotient of 57 patients included in the detailed evaluation remained stationary in 11 cases, rose in 17 cases, and fell in 29 cases. The author notes that the last category includes "falls due to normal average yearly de-

crease," without providing any data which could be used to correct the raw figures. In this instance it is perhaps more obvious than in the discussion of the other criteria, indicating a slight or moderate but positive effect of the thiamine therapy, that a control group is essential for an intelligent interpretation of the results.

In summary, expressing the differences between the number of improvements and the number of deteriorations as the percentage of the number of cases studied, the following figures are obtained: +4.3 per cent for behavior in lodges, +10.8 per cent for work on school reports, +20.0 per cent for social age, and −21.0 per cent for measured intelligence. The greatest improvement took place in a criterion which contains a large amount of subjective element. From the description of the experiment, one is not certain that the improved social age was not a result of the greater attention the patients have received as a result of participating in the experiment. At any rate, the difference between the mean social ages of the group on the two occasions is negligible. The results of intelligence measurements, which appear to be basic to the argument, do not lend support to the thesis that mental deficiency can be treated, with a dependable measure of success, by giving the patients oral supplements of 3 mg. of thiamine daily for five to six months.

In view of the lack of full understanding of the etiology of mental deficiency and mental illness, and the pressure—moral and social—toward therapy, studies such as Rudolf's have their justification, but it should be clear that the study represents a "shot in the dark" approach to therapy. No effort was made to characterize the nutritional status of the subjects, especially as far as thiamine is concerned, at the beginning or during the course of the treatment, and no indications of inadequate thiamine intake or utilization were given. The rationale of the study was not stated satisfactorily, R. F. Harrell's success in "improving" normal children's intelligence (*Nutrition Reviews* **4,** *343 (1946)*) being the main support.

This is not to imply that studies on the relation between nutrition on one hand and intellect and personality on the other hand do not deserve attention. Particularly in the vast number of cases of malnutrition in the tropical regions of the world, disturbed metabolism due to faulty nutrition and its profound influence on behavior constitute an important but hardly tapped area of research (J. N. P. Davies of Uganda, E. Africa, *personal communication*).

Important neurophysiologic aspects of nutrition must be considered in terms of enzyme chemistry. It is true that such physiologic phenomenon as conduction of nerve impulses and learning appear at first too far removed from the simple chemical reactions studied by the chemist in isolated enzyme systems. But the identification of thiamine, the antineuritic vitamin, as a part of the coenzyme of carboxylase (R. R. Williams, R. E. Waterman, and J. C. Keresztesy, *J. Am. Chem. Soc.* **56,** *1187 (1934)*), points out its role in carbohydrate metabolism of the nervous tissues. R. A. Peters (*Lancet* **I,** *1161 (1936)*; *Nutrition Reviews* **3,** *186 (1945)*) observed earlier that the metabolism of the brain tissues from thiamine-deficient pigeons is impaired, showing a somewhat lower oxygen uptake and a slightly greater concentration of lactic acid than found in the brain from normal pigeons. Researches on the role of thiamine in tissue metabolism were reviewed in detail by B. C. P. Jansen (*Vitamins and Hormones* **7,** *83 (1949)*).

A decreased enzyme activity resulting from inadequate vitamin supply constitutes a chemical lesion which in turn brings about physiologic and pathologic signs of deficiency. The general usefulness of the concepts of enzyme chemistry in the elucidation of physiologic processes was appraised by W. E. Knox (in "Research in Medical Science," edited by D. E. Green and Knox,

p. 137, Macmillan, New York (1950)). In time, fundamental advances in the solution of at least some of the phenomena of mental dysfunction may be expected through brain enzyme studies (W. Ashby, *Am. J. Psychiat.* **106,** *491 (1949)*).

NUTRITION AND PUBLIC HEALTH

To increase the interest of both state and local health agencies in the possibilities for public service along nutritional lines and to facilitate dissemination of nutrition education to the medical practitioner and lay population, the Food and Nutrition Board of the National Research Council established a Subcommittee on Diagnosis and Pathology of Nutritional Deficiencies. The report of this subcommittee on nutrition programs in the state health departments has been published (*Pub. Health Reports* **65,** *411 (1950)*).

Based on recent advances in experimental and applied nutrition, there is a growing appreciation of the social, economic, and political significance of nutrition. Indications of inadequate nutrition among certain large segments of the population warrant a more active role on the part of state and local health departments in the accurate diagnosis of such deficiencies, and assessment of their prevalence. Inasmuch as many pathologic states can be attributed to malnutrition, the ultimate program should encompass the entire population. With amplification of the needs in the direction of accurate diagnosis as well as of education in the principles of nutrition, it becomes obvious that leadership in the over-all program should be in the hands of physicians who are specially trained in public health nutrition.

Nutrition service in state health departments had its beginning before the turn of the century in food control activities. Later the studies on pellagra emphasized the importance of nutrition in the public health program. Another step forward was the activity in various states in the study of child hygiene, culminating in the larger plans for improving maternal and child health. Before 1920 only two state health departments employed nutritionists, but after that time such a position was provided for in most of the states and supported increasingly by federal funds. One great difficulty still resides in the fact that salaries for well-trained nutrition personnel are not high enough to compete with other agencies employing high-caliber nutritionists.

Qualifications for public health nutritionists have been established but the number of qualified nutritionists available is limited by the facilities for advanced academic training and for guided field experience. Although in most states the nutrition service is administered through the maternal and child health bureau, nutritionists are increasingly giving help to many other divisions of the health agency, notably in connection with services to adults. Thus, consultation and education continue to be major activities though there have been shifts in demand since the war. Also, in those states in which the nutrition unit has adequate leadership or satisfactory contacts with facilities, research is assuming greater prominence.

In outlining the activities of a state health department, it was pointed out that the many aspects of the nutrition problem call for application of different skills and interests and it is the responsibility of the state health department to see that available programs and facilities are adequate to protect and promote the nutritional health of the people. The activities may be directed at (1) serving the general population, (2) members of specific groups, or (3) individuals or families.

On the first level, it may deal with mat-

ters of broad policy regarding food production and control, with programs, and with administration. Research in surveying nutritional status and in application of the newer knowledge of nutrition to problems thus uncovered, as well as establishment of laboratory facilities, are also included. Again it may effect professional education of staff members, physicians, and supervisors of food services and provide actual information service with respect to diets and nutritional standards. Regarding the second type of activity, the state health department may provide professional and administrative guidance in the school lunch program, in industrial nutrition programs where opportunity for education also exists, and in the feeding programs in various public institutions where manifold problems challenge the skill of the nutrition worker. Opportunities for nutrition service also exist in teaching groups having similar interests and problems as regards nutrition, such as pregnant women, those attending well-baby clinics, and various clubs. In the third category, the state health department would probably have less direct contact but rather would be available for education and guidance through local groups.

Inevitably the item of priority in the nutrition program of a state health department presents itself. The director of the program brings, in greater or less degree, a broad knowledge of public health and nutrition; in equating the need with the available facilities, he must exercise mature judgment and motivate it with administrative ability.

In the conduct of the affairs of the nutrition activities of a state health department, there are areas of activity for persons with different skills such as nutritionists, physicians, and biochemists. A physician, trained in both public health administration and in clinical nutrition, is in a position to administer, direct, coordinate, and interpret the varied activities of his unit which include, among many others, the broad evaluation of the nutritional status of the population of the state and the recommendation of legislation to carry out programs to improve the nutritional status. The director must be able to appreciate the ways in which research would benefit the nutrition program and be able to elicit the help of groups best qualified to assist in the projects. Needless to say, he must be prepared to exploit effectively the accomplishments of his unit to various lay and professional groups.

The nutritionist has had a highly important part in the development of nutrition programs in state health departments. She has been, and in many states still is, the only person on the staff with special training in nutrition. The qualifications for the post have been well considered and defined and the position involves duties of wide scope. In addition to the skills in the fundamentals of nutrition, resourcefulness is needed to integrate and promote the general program, particularly if there is no physician as director. Where the director is a physician, the work of the nutritionist is complementary to his duties; importantly involved in this is the work of the nutritionist in keeping the unit abreast of the advances in experimental and clinical nutrition. Another important function of the nutritionist is to integrate the work of the state health department with the activities of the extension service and departments of agriculture and education of the state universities.

A public health program on the state level for evaluation and improvement of nutrition should include a combination medical, dietary, and biochemical approach. It should be organized as a major division in the framework of the health department. In states lacking resources for such an organization, other arrangements should be made for services and consultations, calling upon civic groups, professional associations, and other state departments, but under the guidance of a high-ranking person in the organization of the state health department.

DIETARY TREATMENT OF LIVER DISEASE

Emphasis has been made repeatedly in this journal upon the lack of data on the specific requirements of nutrients, especially of lipotropic substances, in the treatment of liver disease in man (*Nutrition Reviews* **6,** *298 (1948)*; **8,** *13 (1950)*). In the latter of these two reviews the studies of G. Klatskin and R. Yesner were discussed. In this investigation 14 individuals with Laënnec's cirrhosis were treated by complete bed rest, alcohol withdrawal, and a basic diet. Improvement in clinical, laboratory, and histologic aspects of liver disease was observed in almost all of the patients. The diet was simply an adequate one containing usually close to a gram per kilogram of protein per day and composed of the usual foods. The food was well eaten by all but 2 of the patients. The authors concluded that improvement was so satisfactory with these three therapeutic measures alone that it hardly seemed necessary to provide supplements to the diet.

In a further effort to separate the various factors responsible for improvement in cirrhosis of the liver, R. D. Eckhardt *et al.* (*J. Clin. Invest.* **29,** *227 (1950)*) studied 3 patients with fatty livers. All 3 patients were chronic alcoholics of long duration. The basic plan of the experiment was to provide a protein-free diet during an initial period and follow this by a nutritious diet containing somewhat more than a gram per kilogram of protein in the form of good food. The diet contained between 30 and 100 mg. of choline daily, and a multivitamin capsule containing vitamins A, C, D, B_1, B_2, B_6, niacin, and pantothenic acid was given daily. Frequent liver biopsies were performed and suitable liver function tests were done repeatedly. In one of the patients, for six days in the middle of the protein-free period, 12 g. of choline were administered daily. Nitrogen balance was followed throughout the studies on each of the 3 patients.

All 3 patients had elevated serum bilirubin levels on admission and evidences of disturbances of liver function. The liver was remarkably enlarged and tender in all 3 patients. During the period of administration of the protein-free diet, which was eight, eleven, and thirteen days, respectively, in the 3 patients, there was a progressive decline in the serum bilirubin. The other liver function tests showed a progressive change toward normal. The liver size decreased and liver tenderness disappeared. Negative nitrogen balance continued throughout this period in each of the patients. Thus clinical improvement of marked degree occurred despite provision of a diet devoid of protein and essentially devoid of lipotropic substances. In contrast to this clinical improvement, evidence of improvement of the pathologic changes in the liver was not seen during the protein-starvation period. The liver tissue was stained for fat, pentose nucleic acid, glycogen, and alkaline phosphatase as well as for routine pathologic examination. There was no essential change in any of these histologic or histochemical studies in the liver during the period of administration of the protein-free diet except in the third patient, in whom biopsies taken just before and just after administration of the choline for six days did show a moderate decrease in fat content in this period.

Following administration of protein, positive nitrogen balance occurred in all 3 patients so that a considerable portion of the protein administered was retained. Improvement in liver histology was dramatic in that fat decreased in amount and the liver cells began to collect pentose nucleic acid in the cytoplasm which was taken as a measure of the protein content of the liver cells.

Despite the dramatic histologic improvement during administration of the nutritious diet, clinical improvement was not nearly

as dramatic as in the protein-free period, largely because clinical improvement had already occurred almost maximally during the first period so that little further clinical or laboratory improvement could be expected.

The authors concluded that improvement in clinical status and liver function could occur despite a negative nitrogen balance occasioned by diets essentially devoid of protein. Liver histology, however, did not improve with such a diet except during the period of choline administration in one patient. This latter fact is not surprising considering our knowledge of the effect of protein-free diets, protein-rich diets, and the administration of choline to animals which developed fatty livers due to dietary deficiency.

The divorcement of clinical findings from those demonstrated by liver histochemistry on the biopsy specimens is difficult to interpret. It is possible that a small amount of fat was mobilized from the liver in these patients even though the biopsy specimens did not indicate this to be histologically significant, or that such simple regimens as bed rest and abstinence from alcohol contributed their part to the clinical improvement that was observed. There is no question but that further studies will be necessary to clarify this problem; nevertheless it is apparent that caution is necessary in assessing the efficacy of a single therapeutic agent since clinical improvement can be observed simply by putting the patient to bed and withholding alcohol. These results are much the same as those reported by Klatskin and Yesner (*loc. cit.*), except that in the present study protein was completely withheld during a preliminary period.

MINERAL NUTRITION IN RATS

In considering the dietary requirement for minerals, it is important to know how the metabolism of one mineral may be influenced by others. Thus, iron cannot be utilized by animals for hemoglobin formation unless copper is also supplied in the diet. This interesting mineral relationship was demonstrated by E. B. Hart and associates in 1928 (*J. Biol. Chem.* **77,** *797 (1928)*).

Evidence has accumulated that certain minerals, in excess quantity, may interfere with the normal functions of copper in nutrition. A diarrhea-like condition in cattle, called "teart," reported by W. S. Ferguson, A. H. Lewis, and S. J. Watson (*Nature* **141,** *553 (1938)*) was associated with an excessive molybdenum intake that interfered with effective utilization of copper.

A condition in sheep described as "sway-back" is also considered to be a copper deficiency disease and is precipitated by consumption of forages that contain more than average amounts of lead and zinc.

Thus, at least three elements, molybdenum, lead, and zinc, have been implicated as being harmful in nutrition by interfering with normal utilization of copper in animals. A study of the interrelationships of these elements in the nutrition of the rat was undertaken by L. F. Gray and G. H. Ellis at the U. S. Plant, Soil, and Nutrition Laboratory at Ithaca, New York (*J. Nutrition* **40,** *441 (1950)*).

The determination of molybdenum toxicity in the rat was undertaken in the first experiment. Two types of diets were used, a powdered whole milk diet and a commercial rat chow. The powdered milk diet was mineralized to contain 6.3 micrograms of copper, 37.5 micrograms of iron, and 100 micrograms of manganese per gram. The commercial rat chow contained 14.1 micrograms of copper, 406 micrograms of iron, and 88.6 micrograms of manganese per

gram. The diets thus differed considerably with respect to iron and copper content. Molybdenum was added to the diets at the following levels: mineralized milk diet—0.00, 0.04, 0.08, and 0.12 per cent; rat chow—0.00, 0.04, 0.10, and 0.40 per cent.

Molybdenum toxicity was observed on both basal diets and weight gains were progressively less as the dietary concentration of this element increased. Rats receiving either diet plus 0.04 per cent molybdenum appeared normal. At higher levels of molybdenum the coats of the rats were often rough and the hair remained short and fine like that of young rats. The rats receiving the chow plus 0.40 per cent molybdenum were all dead after a three-week experimental period. The hemoglobin levels remained within the normal range on all levels of molybdenum tried.

To determine whether copper would have a curative effect in molybdenum-poisoned rats, copper supplements of 0.20 and 0.40 mg. per rat per day were tried. The rats which received the milk diet plus 0.08 per cent molybdenum for seven weeks responded well to either level of copper supplement during a subsequent five-week experimental period. The gains of the rats on the higher level of copper were twice those of controls which were continued on the same diet without copper supplement. Copper supplements were without effect in the animals receiving the milk diet plus 0.12 per cent molybdenum or the chow diet plus 0.10 per cent molybdenum.

After the toxicity of molybdenum was established, a factorial experiment was designed which permitted a critical evaluation of the effects of all possible combinations of copper, molybdenum, zinc, and lead as supplements to the mineralized powdered whole milk diet. Eighty rats were used, or a total of 5 rats to each treatment. The mineral levels selected were 0.01 per cent copper, 0.08 per cent molybdenum, 0.50 per cent zinc, and 0.005 per cent lead. From the previous study it was decided that 0.01 per cent copper would be sufficient to correct the toxicity resulting from 0.08 per cent molybdenum.

Growth retardation was noted in all rats that received the diet plus molybdenum. A combination of molybdenum and zinc produced more pronounced growth inhibition than molybdenum alone, whereas zinc by itself did not inhibit growth. Lead had no influence on growth, either alone or with all combinations of the other elements studied.

Contrary to expectations, the 0.01 per cent level of copper failed to correct the growth-depressing effects of 0.08 per cent molybdenum. This is difficult to understand since a dietary level of 0.01 per cent copper must have represented a considerably higher intake than the supplement of 0.40 mg. of copper per rat per day that was observed to have a curative effect in molybdenum-poisoned rats (milk diet plus 0.08 per cent molybdenum) reported in the first experiment.

Hemoglobin determinations were made at the end of the seventh and eighth weeks. A significant lowering of hemoglobin was observed in all rats receiving zinc alone or zinc in combination with either molybdenum or lead, or both. The lowest hemoglobin levels occurred in rats receiving both zinc and lead. The zinc-induced anemia was overcome with copper. Copper appeared to be least effective when molybdenum was also present in the diet.

The effect of molybdenum in retarding rat growth is a confirmation of results reported by J. B. Neilands, F. M. Strong, and C. A. Elvehjem (*J. Biol. Chem.* **172,** *431 (1948)*) and C. L. Comar, L. Singer, and G. K. Davis (*Ibid.* **180,** *913 (1949)*). The latter workers also noted that molybdenum toxicity was much less pronounced in mature than in young rats. The molybdenum-copper relationship could be expected to be somewhat different in mature rats compared to young rats because the requirements for copper are considerably greater in the young than in the mature rat.

The anemia-inducing action of excess dietary zinc was reported in 1937. A growth-depressing action of zinc has also been reported (*Nutrition Reviews* **5,** *7* (*1947*)). The growth-depressing action could be overcome with liver extract, whereas copper was needed to correct the anemia.

Of obvious importance is the question of whether elements such as lead, zinc, or molybdenum are essential in the nutrition of animals. Zinc is a constituent of an enzyme, carbonic anhydrase, and therefore undoubtedly is a dietary essential for man as well as for other animals (*Nutrition Reviews* **1,** *411* (*1943*)). W. R. Todd, C. A. Elvehjem, and E. B. Hart (*Am. J. Physiol.* **107,** *146* (*1934*)) noted that zinc accelerated the growth of rats on a diet low in this element. It should be emphasized that the level of dietary zinc required for normal nutrition is considerably below levels that produce toxic reactions.

Whether lead or molybdenum can be considered essential elements in animal nutrition has not been established. The latter is essential in plant nutrition.

It is becoming increasingly apparent that in the mineral nutrition of the rat, at least, relationships exist between copper and zinc, copper and molybdenum, zinc and molybdenum, and probably between lead and zinc. With these relationships in mind, it becomes obvious that specific mineral requirements for animals are difficult to define. Deficiencies may result with adequate intakes of a given element, such deficiencies being precipitated by the presence of other elements.

IMPROVEMENT OF NUTRITIVE VALUE OF CORN BY PLANT BREEDING AND SELECTION

During recent years, corn has been intensively studied to find methods of increasing the relative amounts of certain constituents having nutritional significance. Oil is the most valuable product of the corn milling industry, per unit of weight. It is useful to the animal being fattened on corn, and processed corn oil enters largely into the human diet. Most of it comes from the embryo of the seed, rather than from the endosperm. The small amount found in the endosperm is not recoverable and sometimes interferes with starch extraction. Oil content differences are mainly varietal ones. High and low strains vary from 1.6 to 12.0 per cent oil.

A. M. Brunson, F. R. Earle, and J. J. Curtis (*J. Am. Soc. Agron.* **40,** *180* (*1948*)), analyzed fifty-seven ears from F_2 plants from various crosses. They dissected the kernels to bran, germ, and endosperm fractions, which averaged 1.7, 41.0, and 0.9 per cent oil, respectively. On the whole kernel basis, the corresponding average oil contents were 0.1, 6.7, and 0.7 per cent. Greatest variability was found among the germ fractions. Germ oil content in the whole kernel and the total oil in whole kernel are highly correlated, showing that whole grain analyses for oil would be satisfactory as a basis for selection in a breeding program aimed at increased oil content.

G. F. Sprague and B. Brimhall (*Agron. J.* **41,** *30* (*1949*)) showed that the effect of season on oil content variation is small when compared with the effect of parentage. They studied four crosses between inbreds and found that the germ content of the kernel varies linearly with the percentage of oil in the kernel, while the oil content of the germ increases as a curvilinear function of the percentage of oil in the kernel. These workers calculated that at least twenty genes are involved in the inheritance of oil content.

Later they studied the relative effectiveness of recurrent selection versus selection during inbreeding on the oil percentage in

the corn kernel (*Agron. J.* **42,** *83* (*1950*)). At the end of a five-year period, the former procedure was 2.6 times more efficient than selection during inbreeding. They would expect that the relative efficiency of recurrent selection would increase as the period of time is increased. This procedure promises to be useful for improvement of cross-pollinated crops in general and can be modified to suit special needs.

Protein is another important constituent of corn, and its quality is especially affected by the amino acid composition. Studies on protein improvement are being pursued by K. J. Frey (*Agron. J.* **41,** *113* (*1949*)). Earlier work at the Illinois Experiment Station extending over many years has given high- and low-protein strains and now their progeny and backcrosses are being studied. Frey concluded that both low-protein percentage (in the kernel) and low-zein percentage are completely dominant. Low contents of valine and isoleucine are also dominant but low-leucine percentage is only partially dominant. Since hybrid vigor affects the non-protein part of the kernel most, this may partially confuse the dominance of low-protein content.

From inheritance studies it was concluded that the minimum number of genes determining the percentages of those materials are for protein 22, zein 6, tryptophan 15, valine 8, leucine 8, and for isoleucine 6. Excepting for protein the number determining each character is relatively small compared to those postulated about other quantitative characters. Protein content may be determined by a relatively small number of genes and a large number of modifier genes because it is still possible to raise the mean of the high-protein parent slightly by selection. The general nature of gene interaction determining these components was found to be arithmetic rather than geometric in the high- x low-protein cross. For future work it appears that the dominance of low-protein content requires that all inbreds going into a high-protein hybrid must be of high-protein content. Thus a new series of inbreds must be isolated or old ones modified. The same would be true for components of protein. These considerations may soon assume added industrial significance because of recent developments in improved synthetic fibers made from corn protein.

The nutritional deficiency of corn protein is in part due to absence of tryptophan and lysine in zein, the alcohol-soluble major protein constituent. Zein and total protein contents are positively correlated. The non-zein protein fraction also increases as zein increases. K. J. Frey, B. Brimhall, and G. F. Sprague (*Agron. J.* **41,** *399* (*1949*)) analyzed data from several suitable crosses and report that non-zein protein percentage is a better criterion of tryptophan content in the corn kernel than is total protein percentage. Yield is negatively correlated with high total protein. Since the proportion of zein increases with total protein content, the protein of high-protein strains is nutritionally inferior to that of normal corn.

Some plant breeders seek to improve the quality of corn protein by increasing the non-zein fraction at the expense of zein. The germ protein is relatively high in tryptophan and germ size is easily modified. Such selection might well accompany selection for high-oil content and thus not affect the quality of endosperm protein. Of ten lines studied, experiments designed to change the zein-to-total protein ratio were not effective.

The ingestion of corn is associated in our minds with pellagra, not only because of the geographic distribution of the disease but also because animal and microbiologic assays have indicated that this cereal is particularly low in niacin. There is a welcome surprise, therefore, in the suggestion that corn might be made a good source of this vitamin merely by selection of the best varieties for cultivation. Data presented by P. R. Burkholder, I. McVeigh, and D. Moyer (*Yale J. Biol. Med.* **16,** *659* (*1944*))

indicate that different samples of corn may show up to eightfold variations in their content of niacin. The sample with the lowest value, 7.9 micrograms per gram, would need to be consumed at a level of approximately 2000 g. daily to supply the 18 mg. recommended for man. The sample with the highest value, 62.1 micrograms per gram, on the other hand, ranks with most cuts of meat as a source of niacin. The data of Burkholder *et al.* suggest that the differences between the samples may be caused by genetic factors.

F. D. Richey and R. F. Dawson (*Plant Physiol.* **23,** *238* (*1948*)) made an extensive survey of the possibilities of breeding corn with higher content of this important vitamin. They found a three and a half fold range of niacin contents among twenty-four inbred lines and concluded that corn hybrids with niacin concentrations of 50 micrograms per gram could be developed. J. W. Cameron and H. J. Teas (*Proc. Nat. Acad. Sci.* **34,** *390* (*1948*)) reported twice as much niacin in sugary types of corn as in the starchy types. Exploratory assays indicated moreover that contents of both thiamine and biotin in the endosperm are also higher in the sugary types. It is not known whether the primary action of the genes relates directly or indirectly to the carbohydrates or to any or all of these vitamins.

A study was undertaken by L. D. Rodriguez, C. H. Hunt, and R. M. Bethke (*Cereal Chem.* **27,** *67* (*1950*)) to determine whether a correlation exists in corn-inbred lines between niacin and pantothenic acid contents and between the contents of each of these vitamins and protein. Thirty-nine inbred lines were used. Wide ranges were found as follows: 9.9–17.8 per cent protein, 12.4–54.1 p.p.m. niacin, and 2.6–9.4 p.p.m. pantothenic acid. Correlation coefficients were not significant for 39 pairs, but they closely approach significance at the 5 per cent level for protein with niacin (negative correlation) and niacin with pantothenic acid (positive correlation), and indicate that there is probably some relationship between the components compared.

Considerable work lies ahead in the complete exploration of possibilities in this field. This seems amply justified for such an important crop as corn to our national food supply.

FORMATION AND CIRCULATION OF ASCITIC FLUID

The factors that control formation and resorption of ascitic fluid are still poorly understood although considerable information has accumulated in recent years (*Nutrition Reviews* **7,** *26, 345* (*1949*)).

Investigators at the University of Rochester have been interested for many years in the production, distribution, and metabolism of the body proteins. Much of their work has centered about the production of plasma proteins and the labile exchange between reserve protein stores and the circulating plasma. Since plasma proteins have some importance in formation of ascitic fluid, and since ascites constitutes a considerable drain on the plasma and other protein stores, it was natural that they would be interested in studying experimental ascites.

A method was developed to produce ascites in the dog by placing an aluminum band around the inferior vena cava between the diaphragm and the right auricle so that the lumen of this vessel was constricted to about one-half of its normal diameter (F. W. McKee *et al.*, *J. Exp. Med.* **87,** *457* (*1948*); McKee, J. A. Schilling, G. H. Tishkoff, and R. E. Hyatt, *Surg. Gynec. Obstet.* **89,** *529* (*1949*)). Dogs subjected to this procedure did not develop demonstrable loss of function or pathologic changes of important degree in the abdominal viscera aside from

the changed circulatory dynamics, but did begin to develop ascites within a few days of operation.

In the studies cited above it was shown that the rate of formation and composition of the ascitic fluid varied markedly with alterations in diet, the most important factors being protein and salt. High-protein diets tended to decrease ascites although the protein content of the ascitic fluid increased at times. Sodium chloride, added to either a high- or low-protein diet, markedly increased the accumulation of ascitic fluid, increased the amount of protein lost in this fluid, and caused a depression of the circulating plasma proteins. Much of the calculated plasma protein loss could be accounted for in the ascitic fluid. The least ascites occurred when high-protein, low-salt diets were fed. Water could be given freely without depression of plasma proteins or increased ascites so long as the sodium intake was low. These results are reminiscent of observations made on human patients suffering from ascites secondary to cirrhosis of the liver (*Nutrition Reviews* **7,** *345 (1949)*).

F. W. McKee and associates (*J. Exp. Med.* **90,** *447 (1949)*) have continued their work by studying the influence of other substances and procedures such as intravenous plasma, ascitic fluid, and amino acid mixtures. The results were not entirely what was expected. Since a high-protein diet tended to maintain normal plasma protein concentration and decrease ascites, it was expected that dog plasma or ascitic fluid administered intravenously would likewise check ascites. However, the reverse was found.

Heparinized pooled dog plasma obtained from normal donor dogs was given to a dog which was producing abundant ascitic fluid on a low-protein salt-supplemented diet. Plasma (350 ml. daily) given by vein plus 50 g. of glucose and a vitamin mixture in 300 ml. of water by mouth raised the plasma protein level from 4.1 g. per cent to 5.9 and 7.0 g. per cent in successive weeks. Despite these high levels of circulating plasma protein, production of ascitic fluid remained high and the level of protein in the ascitic fluid doubled and quadrupled. More than two-thirds of the administered protein was found in the ascitic fluid. When intravenous plasma protein was discontinued, ascitic fluid production dropped to a very low level and plasma protein increased slightly. Similar experiments in 2 additional dogs gave almost identical results. Comparison of the analysis of ascitic fluid and circulating plasma showed very similar albumin-globulin ratios although the level of all proteins in the ascitic fluid was lower. Electrophoretic patterns of circulating plasma and ascitic fluid were also similar. It was evident that globulin crossed into the ascitic fluid as easily as albumin under these experimental conditions.

In other experiments an amino acid mixture, which was known to favor production of blood proteins in dogs, was given intravenously. When these amino acids were given in distilled water there was a prompt rise in circulating plasma protein and a reduction in ascites formation. On the other hand, if the amino acids were given in normal saline, ascites formation increased and plasma proteins were depressed.

Ascitic fluid obtained from dogs with ascites was given intravenously to 2 dogs. A large outpouring of ascitic fluid resulted with a drop in circulating plasma proteins. These dogs lost as much or more protein in the ascitic fluid as was given intravenously. Ascitic fluid, added to a low-protein diet, was given to 2 dogs. A sharp and remarkable drop in the circulating plasma protein level with abundant ascites resulted. The drop in plasma proteins was greater than would have been expected from a low-protein diet with added salt.

The anomalous behavior of intravenous plasma and of both oral and intravenous ascitic fluid, whereby ascites actually increased even though protein was added rapidly to the blood, can be explained in part

on the basis of sodium chloride. The amount of sodium in the plasma and ascitic fluid was not determined but was undoubtedly present in considerable amounts. Whether the amount of salt was enough to account for all of the effect is not certain. The authors believe that salt is only a part of the explanation. This is particularly true of the experiments in which ascitic fluid was given orally. They believe that ascitic fluid contains a factor in addition to salt which favors production of ascites. In any event it is clear that simply increasing the plasma protein level, or using measures which would be expected to increase the plasma proteins, would not diminish ascites unless sodium chloride was simultaneously restricted. It would have been interesting to observe the effect of plasma and ascitic fluid proteins which had been largely freed of salt. Such experiments are presumably planned. Salt-poor serum albumin has been used clinically in treating ascites secondary to cirrhosis of the liver. In some instances increasing the plasma protein level in this fashion did result in clearing of ascites, but in other instances ascites remained despite normal or near normal concentrations of protein in the blood, and of simultaneous sodium restriction (*Nutrition Reviews* **7,** *345 (1949)*).

Several observations made in the course of the above experiments suggested the possibility that protein present in ascitic fluid might interchange with blood plasma proteins to an extent not generally considered possible. Ascitic fluid as it accumulates in man and animals has been considered as a stagnant pool that contains protein which is relatively inert metabolically in the sense that it is not an active part of the body protein pool. Direct evidence on this point has been lacking. The University of Rochester group has now supplied evidence that there is a rapid exchange of protein between ascitic fluid and plasma, at least under the conditions of their experiments. They have called this phenomenon "ascitic fluid circulation" (F. W. McKee, W. G. Wilt, R. E. Hyatt, and G. H. Whipple, *J. Exp. Med.* **91,** *115 (1950)*). Dogs with ascites produced by inferior vena cava constriction were used as before. A high-protein diet consisting of 200 g. of cooked horse meat plus 50 g. of a standard mixture of lard, sucrose, cod liver oil, yeast, and bone ash, and 4 g. of sodium chloride daily were used to insure continuous formation of ascitic fluid of high-protein content throughout the experimental period.

The movement of protein was followed using a radioactive tracer technic. Radioactive plasma was produced by feeding lysine, labeled with C^{14} in the epsilon position, to a donor dog, subsequently withdrawing blood and separating the plasma. This procedure had been shown to result in plasma in which most of the radioactivity was in the plasma proteins (L. L. Miller *et al., J. Exp. Med.* **90,** *297 (1949)*). This plasma was then injected intraperitoneally, after removal of an equivalent amount of ascitic fluid, into 2 dogs with ascites. Blood samples were taken at frequent intervals for forty-eight to seventy hours, and at the end of this period the ascitic fluid was removed as completely as possible. Radioactivity measurements on the blood samples and on the residual ascitic fluid permitted an estimate to be made of the degree to which the plasma and ascitic fluid proteins interchanged. The results indicated that between 35 and 40 per cent of the radioactive plasma was absorbed into the general circulation within the experimental period. Of the protein absorbed, one-third to one-fourth was found in the circulating plasma, the rest presumably was in extravascular and tissue proteins, or perhaps some had been excreted. It should be emphasized that this result was obtained despite continuous formation of ascitic fluid, *i.e.*, the predominant flow of protein was from plasma to ascitic fluid.

In another ascitic dog, radioactive plasma was given intravenously. In this instance at least 31 per cent of the injected plasma was transferred into the ascitic fluid, 11 per cent

remained in the blood, and 58 per cent was presumably in the extravascular protein stores. This result would have been expected in view of the continuous formation of ascitic fluid.

These data seem to show clearly that there is a rapid exchange of protein between plasma and ascitic fluid and indicate that ascitic fluid turnover may be complete in four to seven days. However, the figures cited above must be regarded as only approximating the rate at which this exchange occurs. If there is a dynamic equilibrium between plasma and ascitic fluid then some of the protein must have been exchanged several times and would not add to the final percentage figures. Also, the administered plasma must have contained an unspecified amount of radioactivity in the nonprotein fraction. These smaller molecules should be more freely diffusible than the protein molecules. Although the amount of radioactivity in the nonprotein fraction was not reported in these experiments, it can be inferred from previous work (Miller *et al.*, *loc. cit.*) that this source would be insufficient to explain all the radioactivity interchange.

The extent to which the findings in these interesting experiments are applicable in understanding human ascitic fluid formation is uncertain. Ascites in these dogs differs in many important particulars from human ascites. Nevertheless, these experiments do provide a clear demonstration that rapid interchange of protein does occur in dogs, and indicate the possibility of a similar phenomenon in human disease. Furthermore, it seems certain that if large protein molecules can interchange so rapidly then smaller molecules such as water and electrolytes may exchange very rapidly.

G. J. Vosburgh *et al.* (*Am. J. Obstet. Gynec.* **56,** *1156* (*1948*)) have shown that another compartmented fluid pool, the amniotic fluid, is not an inactive stagnant pool, as had been thought. Using an isotope tracer technic they were able to show that the water of this fluid was replaced completely about once every three hours. Sodium was completely replaced about every fifteen hours.

It seems probable that most, if not all, of the body fluids and their constituents are in an active dynamic equilibrium to an extent which is only beginning to be realized. The factors which govern these equilibriums are still incompletely understood not only in disease states but in the normal as well. It is clear that the older hypotheses advanced to explain the mechanism of fluid formation are now inadequate.

HEPATIC CHANGES IN PIGS RESULTING FROM A COLD ENVIRONMENT

In 1924 M'Gowan of Aberdeen, Scotland, published a paper in which he attempted to explain the anemia noted in suckling pigs raised in that area. Anemia was observed in the litters of sows which had been placed under cover in concrete pens for a fortnight before bringing forth their young. The disease usually appeared during the second and third weeks of life and was fatal, the animals often dying suddenly. Postmortem examination, as a rule, disclosed advanced anemia, dropsical effusion into the various body cavities, and great enlargement of the heart, as well as changes in the center lobules of the liver (J. P. M'Gowan, *J. Path. Bact.* **27,** *201* (*1924*)). It also was noted at that time that anemia could be prevented by administration of ferric oxide in food given to the sow. In fact, the disease could frequently be prevented in this manner. However, many of the animals, especially if they had been affected before treatment was commenced, never did well; they ceased to grow, remained permanently stunted, and were affected by a chronic cough.

It seems well established that if piglets

are reared on pasture from birth or have access to soil or sods in concrete pens they do not become anemic (H. C. H. Kernkamp, *J. Am. Vet. Med. Assn.* **87,** *37* (*1935*)). Further, it has been demonstrated that spontaneous recovery from this anemia commences at the time of weaning, providing the anemia has not been so severe as to produce permanent damage.

Though administration of iron relieved the anemia in suckling pigs, it apparently remained very difficult to rear healthy pigs indoors in northeastern Scotland during the colder months of the year. Pigs reared in small wooden ark huts on pasture did obtain good weights and appeared healthy when weaned at 8 weeks of age. This difference between pigs raised outdoors and those raised indoors has been the subject of investigation by J. M. Naftalin and J. W. Howie (*J. Path. Bact.* **61,** *319* (*1949*)). Howie, W. A. Biggar, W. Thomson, and R. Cook (quoted by Naftalin and Howie, *loc. cit.*) had previously noted that the complete sequence of events related to anemia of suckling pigs which M'Gowan attributed to a deficiency of iron could not be prevented by giving a large dose of iron by mouth. On the other hand, these events could be prevented if the young pigs were given, in addition to iron a warm, dry, and comfortable environment instead of a damp, cold area built of concrete. Howie felt that the comforting warmth, rather than the outdoor soil and grass, was the reason for the superior health of the pigs. The current study was undertaken to furnish a detailed description of the pathologic changes in the livers of young pigs reared in a cold and damp environment and to determine whether any histologic change might throw some light on the pathogenesis of this peculiar disease.

Nine litters of pigs (67 piglets) were allocated to one of three groups, according to the type of housing to be utilized. One group was placed in open pens in a cold, drafty, and often damp byre with an uninsulated floor and only a limited amount of straw for bedding. The second group was placed in a warm shelter provided by a wooden ark hut or an electric hover. The third group was put in wooden ark huts which provided shelter similar to that for group 2, but the pen had an outdoor concrete run adjacent to the byre in which groups 1 and 2 were housed. Sixteen of the 30 pigs in group 1 died; only 5 of 21 pigs in group 2, which was warmly housed, died; and there were no deaths among the 16 pigs in group 3, which was protected by housing that was warm and the animals had access to the open air but not to soil or grass.

At necropsies of the 21 pigs, attention was devoted mainly to the liver, which showed the most obvious lesions. All animals had received 1 g. of reduced iron by mouth on the third, tenth, and seventeenth days of life. This amount among pigs which are comfortably housed is sufficient to prevent a fall in hemoglobin to anemic levels.

All animals were in good health up to 3 weeks of age, but great differences were noted clinically in the animals housed in the cold and in the warm environments. The pigs in group 1 (the coldest environment) were noted to have anorexia, which seemed to develop about the seventeenth day of life. Pigs in groups 2 and 3 remained playful, whereas those in group 1 lost this quality. It was often observed by the writers that an ever-changing small number of piglets from the cluster of pigs in group 1, which lay with their abdomens pressed to the floor, would disturb the other pigs, force their way out, run around a little, and then try to climb on the backs of the pigs remaining in the cluster. As a result, the group was rarely at rest. On the other hand, in groups 2 and 3 the pigs lay quietly and snugly on the floors of their huts but not pressed into a tight cluster. Abdominal distention soon developed in the pigs in group 1 and, of the 21 pigs in this group which were examined, 13 had ascites. The hair of the animals had grown long and lacked lus-

ter, the tips of the ears were blue, and many of the animals had intermittent diarrhea.

The two consistent findings described were ascitic fluid and gross changes in the liver. The livers were usually enlarged and enlargement of the lobules was visible on both external and cut surfaces. Histologically, the livers showed essentially the same type of lesion in each animal. These components consisted of vascular congestion, the sinusoids being greatly distended with red cells; escape of red blood cells into the tissue spaces; fatty changes in the liver cells; fragmentation of columns of liver cells and necrosis of individual cells; and fibrosis. These types of changes are seen in human beings who have increased venous pressure.

From this study it seemed obvious that the blame for all of the changes observed could not be placed on a deficiency of iron, for the test animals were provided with an amount of iron sufficient to prevent a fall of hemoglobin under normal circumstances. The authors speculated as to whether these changes could be the direct or indirect result of exposing the pigs to excessive cooling. They pointed out that the young pigs were poorly protected against cold and that they were only able to prevent this disease when a wooden ark hut was added to the pigs' environment. It is well known that continued cold will constrict the peripheral vessels, driving blood into the systemic circulation and, thus, raising venous pressure. B. Maegraith, W. H. H. Andrews, and D. Gall (*Lancet* **II,** *781 (1947)*) have emphasized the importance of obstruction to the flow of blood arising from changes in the hepatic vessels themselves and not necessarily from cardiac failure. It is also well known that the hepatic venous tree of pigs contains a considerable amount of muscle, and it is not difficult to imagine that cold, especially that applied directly to the abdomen as happens in a young pig lying on an uninsulated, damp floor, might cause derangement of the blood flow through the liver. If damage to the liver is accepted as the main pathologic feature in this disease of young pigs, then this, of course, will explain the other clinical symptoms which the authors have described.

There seemed to be many unanswered questions in this study, which the authors raised themselves. They do not know in what manner prolonged exposure to excessive cooling may act on the nutritional state of the animals. Such cooling will increase the caloric and protein requirements and, perhaps, the need for specific nutrients.

It appears that in this well-carried-out experiment, the changes in the liver are real, and the chances are that the authors' suggestion regarding pathogenesis is correct. More and more the importance of the great changes which can be produced by altering the circulation in the hepatic parenchyma itself is being appreciated. Likewise, the importance of environment in relation to nutritional disorders is again being emphasized.

ESSENTIAL AMINO ACID REQUIREMENTS FOR MAINTENANCE AND FOR REHABILITATION IN RATS

Nine amino acids have been shown to be required by adult rats for maintenance: tryptophan, histidine, lysine, threonine, phenylalanine, methionine, leucine, valine, and isoleucine. A tenth amino acid, arginine, is essential for growing rats but does not appear to be essential for maintenance of the adult rat. P. A. Wolf and R. C. Corley at Purdue (*Am. J. Physiol.* **127,** *589 (1939)*) established these qualitative amino acid needs of adult rats for maintenance, and confirmatory qualitative data were reported by R. W. Wissler and associates at the University of Chicago (*J. Nutrition* **36,** *245 (1948)*).

Workers from the latter laboratory, E. P. Benditt, R. L. Woolridge, C. H. Steffee,

and L. E. Frazier (*J. Nutrition* **40,** *335 (1950)*) and Steffee *et al.* (*Ibid.* **40,** *483 (1950)*), have attempted the next logical step, namely, establishment of the quantitative requirements of the essential amino acids. The first of these two papers deals with the minimum requirements for the well-nourished adult rat. The second paper deals with the requirements for tissue regeneration in adult protein-depleted rats.

These studies were conducted under a standardized set of conditions, including adequate caloric, vitamin, and mineral intakes, and a total nitrogen intake sufficient for maintenance needs.

To find the minimum maintenance level for a specific amino acid, a series of diets was constructed in which all constituents remained constant except the amino acid under consideration. In the first trial the dietary level of the amino acid under test was varied widely in an attempt to bracket the maintenance level. After this approximation had been reached, additional levels were tried in order to establish more precisely the maintenance point. A single animal was used to test a given dietary level of an amino acid except for phenylalanine, where 3 animals were used to test nine different levels of this amino acid.

For several weeks prior to feeding the amino acid diets, the animals were stabilized on an identical diet except that it contained 9 per cent of casein in place of the amino acids.

The test diets were fed for periods of twenty days. Diet consumption, fecal and urinary nitrogen, and body weight changes were used as criteria in establishing the maintenance requirements of the various amino acids.

The mixture of sixteen amino acids used in these studies was patterned after the amino acid composition of casein except that serine, proline, and hydroxyproline were absent. The racemic mixtures of threonine, valine, and isoleucine were contained in the mixture at a level to compensate for the assumed metabolic inactivity of the unnatural D-forms. An amount of glutamic acid, equivalent to these nonutilizable D-forms, was omitted from the mixture. It was assumed that the D-forms of tryptophan, phenylalanine, and methionine were utilized, and histidine, arginine, and lysine were included as the L-forms. No compensation was made for the lower total amino acid content of the diet which resulted when the one under investigation was reduced. The total nitrogen content of 15 g. of the diet, the amount offered daily to rats weighing 300 g., consequently varied from 203 to 223 mg.

The minimum requirement for each of the nine essential amino acids for maintenance of body weight was in every case slightly higher than the minimum requirement for nitrogen equilibrium. The minimum requirements were expressed as milligrams per square centimeter of body surface and these values were then converted to milligrams per kilogram of body weight. Thus, the minimum requirements of the nine essential amino acids for the maintenance of the well-nourished adult rat were (milligrams per kilogram body weight daily) as follows: tryptophan 30, histidine 35, phenylalanine 50, lysine 60, threonine 85, methionine 90, leucine 110, valine 120, and isoleucine 180.

To establish the minimum essential amino acid needs for the rebuilding of body tissues lost during protein depletion, the above values were assumed to be the maintenance needs of protein-depleted animals. Protein depletion was accomplished by feeding adult rats a ration adequate in all respects except protein for two to three months; this depletion resulted in body weight losses of from 24 to 33 per cent. The requirements for effective protein regeneration in the depleted animals were determined by establishing the minimum level of each of the above amino acids which was needed to effect, in ten days, a body weight recovery. Food consumption was also again used as an indicator of the adequacy of the diets. It was felt, however, that weight recovery was a more accurate indicator of amino acid requirements than was food consumption, and car-

cass analyses substantiated this contention. Body weight gains closely paralleled gains in carcass protein.

The quantities of the amino acids considered as minimum required to accomplish body protein regeneration were as follows: (The maintenance values are repeated in parenthesis so that it can be readily seen that there are marked differences in amino acid requirements for maintenance as compared to requirements for the regeneration of tissue protein.) Tryptophan 50 (30), histidine 85 (35), methionine 130 (90), threonine 160 (85), isoleucine 165 (180), valine 170 (120), phenylalanine 205 (50), lysine 270 (60), and leucine 305 (110). The isoleucine requirement for maintenance was found to be greater than that for the regeneration of tissue protein, whereas the phenylalanine and lysine needs for regeneration were four times as great as for maintenance.

The importance of establishing this type of quantitative data can hardly be overemphasized. Information of this type should serve to increase knowledge of the protein requirements of human beings who, as a result of surgical operation, wasting diseases, or other causes often are severely depleted in reserve protein.

The Chicago workers have attempted to establish whether their data obtained with rats may have any resemblance to similar data available for human beings. The amino acid requirements for adult healthy young men were established by W. C. Rose (*Nutrition Reviews* **8,** *118 (1950)*). These data and other calculated data on human requirements were compared with estimated human requirements based on the data obtained with rats. In converting the rat data to man, it was precluded that there is a linear relation between body surface area and the maintenance requirement for essential amino acids.

The quantitative daily requirements in grams of the eight amino acids essential for nitrogen equilibrium in adult healthy men, as reported by Rose, are as follows: (The human requirements estimated by the Chicago workers from their rat data are in parentheses.) Tryptophan 0.25 (0.27), phenylalanine 1.10 (0.47), lysine 0.80 (0.56), threonine 0.50 (0.76), methionine 1.10 (0.79), leucine 1.10 (0.97), valine 0.80 (1.05), and isoleucine 0.70 (1.68).

The requirement of man appears to be higher for phenylalanine and lower for isoleucine than would be suggested from the rat data. Despite these differences the values available for man and those estimated for man from data obtained with the rat are quite similar.

VITAMIN B_{12} AND THE BABY PIG

Several laboratories have reported that baby pigs can be raised successfully by artificial feeding on purified diets supplemented with the known vitamins. The role of vitamin B_{12} in the nutrition of the baby pig fed under such conditions has recently been reported from two different laboratories.

A. L. Neumann and B. C. Johnson, at the University of Illinois, studied the comparative effect of crystalline vitamin B_{12} and antipernicious anemia liver extract (reticulogen) on growth and blood cell formation (*J. Nutrition* **40,** *403 (1950)*). They used 12 48-hour-old pigs and fed a "synthetic milk" ration composed of alpha-protein (an isolated soybean protein) 29.4, DL-methionine 0.6, glucose 30.9, lard 30.8, and mineral salts 8.3 per cent. These ingredients were homogenized into a "synthetic milk" containing 13 per cent solids and vitamins were added as follows (milligrams per liter): thiamine 0.65, riboflavin 1.30, pyridoxine 1.30, calcium pantothenate 7.80, inositol 26.0, niacin 2.6, para-aminobenzoic acid 2.6, choline chloride 130.0, biotin 0.01, folic acid 0.052, ascorbic acid 16.00, alpha-tocopherol acetate 1.00, and 2-methyl-1,4-naphthoquinone 0.26. Vitamins A and D_2 were added at levels

of 2000 and 200 I.U. per liter, respectively. To inhibit intestinal synthesis of vitamin B_{12}, sulfasuxidine was included in the diet at the rate of 2 g. per liter.

After three weeks on this depletion diet, blood and bone marrow examinations were made. Four pigs then received 2 micrograms of crystalline vitamin B_{12} daily intramuscularly, 4 received 0.10 ml. of reticulogen daily intramuscularly, and 4 were continued on the depletion diet as controls. The reticulogen contained 1.9 micrograms of vitamin B_{12} per 0.10 ml. as determined by the *Lactobacillus leichmannii* assay procedure.

The most striking blood abnormality in the vitamin B_{12}-depleted pigs was a high neutrophil count. Fifty to 60 per cent of the leucocytes observed in differential counts were neutrophils in all pigs examined after the initial three-week depletion period. After eight weeks the neutrophil count had increased to 84 per cent of the total leucocytes in the deficient pig examined, whereas values in a vitamin B_{12}- and a reticulogen-treated pig were 20 and 14 per cent, respectively. Lymphocyte values after depletion for three weeks were 34 to 42 per cent. After eight weeks of depletion the deficient pig examined had 15 per cent lymphocytes while the treated animals had 79 to 85 per cent lymphocytes.

There were no differences between deficient and treated pigs with regard to blood hematocrit, hemoglobin, or total red cells.

Among the bone marrow abnormalities noted in the deficient pigs was a decrease in the myeloid cells from a normal of 76 per cent for pigs on natural diets to as low as 20 per cent after eight weeks on the depletion diet. Following vitamin B_{12} or reticulogen, the myeloid cell values returned to normal. The decrease in myeloid cells in the vitamin B_{12}-deficient pigs was accompanied by a corresponding increase in erythroid cells. Following B_{12} or reticulogen therapy, the erythroid cells dropped to 13 and 15 per cent, respectively, as compared to 23 per cent found in pigs on natural diets.

The increase in erythroid cells in the deficient pigs was mainly basophilic normoblasts. Megaloblasts were not found.

Crystalline vitamin B_{12} and reticulogen produced equal growth responses over the five-week assay period. Two of the control pigs died during the experiment and the 2 survivors gained an average of 4 kg. compared to 12 kg. for the pigs receiving either crystalline vitamin B_{12} or reticulogen.

Other abnormalities noted in the vitamin B_{12}-depleted pigs were enlarged and reddened tongues and enlarged livers. The deficient pigs were listless and sluggish in their movements, and posterior incoordination was evident in those that survived the eight-week experimental period. Microscopic examination of the spinal cord and sciatic nerve failed to reveal any degenerative changes that would account for the gross symptoms of incoordination.

To summarize, crystalline vitamin B_{12} was as effective as reticulogen in correcting manifestations of vitamin B_{12} deficiency induced in baby pigs by sulfasuxidine. Notable deficiency signs were an increase in the neutrophils and a decrease in the lymphocytes of the circulating blood, and an increase in erythroid cells with a decrease in myeloid cells in the bone marrow. The deficiency produced in baby pigs and pernicious anemia in man are different in that in the latter disease, a deficiency of granulocytes is characteristic along with lowered blood hemoglobin values. Lowered blood hemoglobins were not observed, and an increase in granulocytes occurred in the vitamin B_{12}-deficient pigs.

The study reported by G. C. Anderson and A. G. Hogan of the University of Missouri is more specifically concerned with the requirement of the baby pig for vitamin B_{12} (*J. Nutrition* **40,** *243* (*1950*)). The ration used was also a vitamin-fortified synthetic milk similar to that used by the Illinois workers, with the following principal changes: (1) it was made up to contain 19 per cent solids, and (2) it contained "vitamin-free" casein as the protein source in-

stead of the alpha-protein of soybean plus methionine.

The casein, according to microbiologic assay, contained 1.7 micrograms of vitamin B_{12} per 100 g. Six 48-hour-old baby pigs were used, 3 of which received intramuscular injections of crystalline vitamin B_{12} every three days, or a total of 50, 100, and 200 micrograms, respectively, over a thirty-eight day experimental period. The remaining 3 pigs served as controls.

During the first six weeks all 6 pigs grew at a uniform rate, which was about equal to currently used growth standards for baby pigs. Following this initial period, the vitamin B_{12}-injected animals began to gain more rapidly than the controls, so that at the end of seventeen weeks the average body weight of the vitamin B_{12}-injected group was 48.8 kg.; one of the control animals had died, and the other 2 weighed 15.9 and 35.6 kg., respectively.

One of the control pigs was declining in weight in the twelfth week of the experiment, had lost its appetite, and had local skin eruptions on the back and sides. This animal completely recovered following vitamin B_{12} therapy. It was given a total of 25 micrograms of vitamin B_{12}, by intramuscular injection, between the twelfth and sixteenth weeks of the experiment, and at seventeen weeks weighed 15.9 kg.

During the first eight weeks the vitamin B_{12}-deficient pigs had intermittent attacks of diarrhea and vomiting. The pig receiving the lowest level of vitamin B_{12} (50 micrograms) also had a severe case of diarrhea during the sixteenth week but completely recovered in three weeks.

The ration was fed in dry form after the tenth week. The pigs receiving vitamin B_{12} adjusted themselves more readily to the dry ration than did the deficient animals. One of the control animals died of acute pneumonia shortly after dry-ration feeding was started. Postmortem examination revealed congestion and fatty metamorphosis of the liver, and extreme congestion and necrosis in the kidneys.

The authors recognize that the amount of vitamin B_{12} required by the pig is of considerable importance and, although their own data on this point are incomplete, they have made what they consider the best estimates that present data will permit. The amount of vitamin B_{12} stored in the tissues of the baby pigs was not known, and no reliable information was available which would permit an accurate calculation of the relative effectiveness of the vitamin B_{12} in the casein compared to that administered by intramuscular injection. With these precluded limitations, the Missouri workers have tentatively estimated the quantitative requirement for vitamin B_{12} to be 0.25 microgram daily per kilogram body weight, when administered orally, or not over 1.5 micrograms per 100 g. of food.

The Missouri workers also included in their study 2 pigs which received cow's milk fortified with 60 g. of sucrose, 2.5 g. of ferrous sulfate, 0.2 g. of cupric sulfate, and 0.02 g. of potassium iodide per liter. At the end of eight weeks the pigs receiving the fortified cow's milk weighed about 50 per cent more than the animals receiving the synthetic milk diet and vitamin B_{12} injections. Explanations offered for the inferior growth obtained with the synthetic milk as compared to cow's milk were that the former may have been partially inadequate, or its physical or chemical composition may have been such as to cause irritation to the alimentary tract.

AMINO ACID EXCRETION IN LIVER DISEASE AND WILSON'S DISEASE

Under normal conditions amino acids appear in the urine in small quantities. The amounts excreted and the renal clearances and other aspects of renal excretion of amino

acids were presented in a symposium held before the New York Academy of Sciences in 1948 (L. D. Wright, *Tr. N. Y. Acad. Sci.* **10,** *271 (1948)*). Similar studies have been reviewed (*Nutrition Reviews* **5,** *213 (1947)*; **7,** *137 (1949)*).

R. D. Eckhardt and C. S. Davidson (*J. Biol. Chem.* **177,** *687 (1949)*) have demonstrated that a normal individual excreted increasing quantities of amino acids with increases in the protein content of the diet on three separate dietary periods, containing, respectively, 0, 75, and 150 g. of protein daily. Nevertheless, an irreducible minimum on a protein-free diet was noted and the change in urinary excretion with alteration in diet was only slight compared to the dietary protein change (*Nutrition Reviews* **7,** *334 (1949)*).

Following intravenous administration, larger quantities of amino acids are excreted than when whole or hydrolyzed protein is given orally (*Nutrition Reviews* **7,** *68, 328 (1949)*). Despite this increased excretion, positive nitrogen balance and evidence of utilization of amino acids for building of body tissues have been observed if sufficient calories and amino acids are given and, of course, if the essential amino acids are present in adequate quantities to meet the minimum requirements (*Ibid.* **8,** *118 (1950)*).

No great deviation from normal was found in patients with liver disease either in the total amino acid excretion or the partition of the essential amino acids (*Tr. N. Y. Acad. Sci., loc. cit.*) Nevertheless, in this study there were considerable individual variations in the quantity of individual amino acids excreted and the number of patients was small (7). It is of course well known that in patients with acute yellow atrophy of the liver, in which this organ undergoes extensive and rapid autolysis, there is marked aminoacidemia and aminoaciduria.

Some indication that there might be an increased excretion of amino acids in liver disease was presented by W. Frankl, H. E. Martin, and M. S. Dunn (*Arch. Biochem.* **13,** *103 (1947)*). Further studies from this laboratory have clearly shown that in many patients with liver disease there may be an abnormal amino acid excretion (Dunn, S. Akawaie, H. L. Yeh, and Martin, *J. Clin. Invest.* **29,** *302 (1950)*). In this investigation the urinary excretion of amino acids was studied in 25 patients, most of whom were suffering from subacute or chronic cirrhosis of the liver. The following amino acids were studied: arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine, and valine. In only a few patients were all of the amino acids determined while in several, determinations of only two or three are listed.

Thirty-eight per cent of the total number of individual urinary amino acid determinations were abnormal, 20 per cent were elevated, and 18 per cent were below the normal. Most of the high values were found in patients with active liver disease and jaundice while the majority of the low values were seen in patients with healing liver disease in whom jaundice was not a notable finding. Three of the amino acids—methionine, tyrosine, and valine—had high excretions in over 50 per cent of the determinations, while almost half of the determinations for lysine and histidine were low, and finally, the majority of the excretion rates for arginine, aspartic acid, threonine, and tryptophan were normal. The authors found considerable difficulty in interpreting the variations in amino acid excretion from normal in liver disease and postulated changes in deamination, conjugation, or utilization as possibilities, although the presence of renal disease could not be eliminated as a factor.

The marked increase in urinary excretion of amino acids found in Wilson's disease (hepatolenticular degeneration) has been

commented upon, although the mechanism was not known (*Nutrition Reviews* **7,** *115 (1949)*). The suggestion was made that the presence of liver disease in this condition might result in decreased deamination of amino acids, and that aminoacidemia and subsequent aminoaciduria would result. Further studies of this syndrome have indicated that the defect accounting for the aminoaciduria may be a renal one (A. M. Cooper, R. D. Eckhardt, W. W. Faloon, and C. S. Davidson, *J. Clin. Invest.* **29,** *265 (1950)*).

Six patients with hepatolenticular degeneration were studied. All had fairly typical neurologic findings. In some of the cases evidences of liver disease were found on physical examination. Laboratory demonstration of liver disease was striking in only one patient, however, although in the others suggestive laboratory evidence of liver disease was present. Liver biopsies did reveal some abnormalities in the 3 patients on whom this procedure was done, but although a slight amount of fibrous tissue was found in one there was no evidence of diffuse hepatic fibrosis or of portal cirrhosis in these 3 patients. It is possible that large bands of fibrous tissue were missed by the small size of the biopsies obtained.

The total average daily alpha-amino nitrogen excretions and the morning fasting excretions were elevated in every determination in each of the 6 patients with Wilson's disease compared to the normal. The plasma alpha-amino nitrogen was well within normal limits and could not account for the increased amino acid excretion, although there were occasional slightly elevated values. Neither urine volume nor total nitrogen excretion could be correlated with amino acid excretion. The excretion of all of the ten essential amino acids was increased in the patients with Wilson's disease as compared to normal. Although as noted above, considerable variation in the excretion of alpha-amino nitrogen and individual amino acids in patients with liver disease has been found, the increases were not nearly as great as those found in the patients with Wilson's disease, despite the fact that the liver disease studied was much more severe than that present in the patients with Wilson's disease. For this reason it was concluded by the authors that impaired liver function could not account for the aminoaciduria in Wilson's disease.

As in liver disease, the blood concentration of amino acids following administration of a complete acid hydrolysate of casein was essentially the same as in normal individuals and, moreover, the increased urinary excretion following amino acid administration was no greater in the patients with Wilson's disease than in normal individuals or patients with liver disease. Finally, the change in blood levels following oral administration of glycine and of glutamic acid was essentially the same in patients with Wilson's disease as in normal individuals, indicating that no defect in the intermediary metabolism of these specific amino acids could be found.

Glucose tolerance tests following oral administration of glucose were normal in the patients with Wilson's disease, although several patients had glycosuria when the blood sugars were normal. This was presumed to be renal in origin.

Evidence is rapidly accumulating that excretion of amino acids in the urine is largely a renal phenomenon unless very high blood levels occur such as after intravenous infusion of amino acids or in acute necrosis of the liver. Thus, even in severe liver disease, although there are some increases in excretion of individual amino acids, they are inconstant and low values are commonly found as well. Moreover, the markedly increased excretion of amino acids seen in Wilson's disease does not appear to be related to diminished deamination of amino acids by the liver, but rather to some type of little understood renal abnormality associated in some instances with glycosuria as well.

NOTES

Vitamin E in the American Diet

Commonly used foods, typified by their availability in Rochester, New York, have been analyzed for vitamin E content by workers in the Research Laboratories of Distillation Products, Inc. (P. L. Harris, M. L. Quaife, and W. J. Swanson, *J. Nutrition* **40,** *367 (1950)*). Analytical methods, which were recently developed by these workers, were used (*Nutrition Reviews* **8,** *157 (1950)*). These methods permit analysis of total tocopherols and the relatively inactive gamma- and delta-forms so that the highly vitamin E-active alpha-form can be estimated by difference. The relatively inactive beta-form has thus far been found only in wheat, and the method for non-alpha-tocopherols could not be applied to wheat or wheat products. However, from bioassay data, it was estimated that from 50 to 60 per cent of the tocopherols in wheat were the highly active alpha-form.

These workers estimated the average per capita consumption of vitamin E from their analytical data and from the average food consumption data compiled for the population of the United States by the U. S. Department of Agriculture. From these calculations, it appears that the average daily consumption of total tocopherols is about 24 mg. About 60 per cent of the total, or 14 mg., was found to be the highly active form, or alpha-tocopherol.

The richest dietary sources of vitamin E are the vegetable oils, which supplied about 60 per cent of that present in the average American diet. Refined cottonseed oil is a particularly rich source of alpha-tocopherol.

Margarine was found to be a good or a poor source depending upon whether it was made from vegetable or animal fats. Since over 90 per cent of margarines manufactured in the United States are made from vegetable oils, principally cottonseed oil, they should, in general, be very good sources of vitamin E.

Cereal grains and their products are generally considered very important sources of vitamin E in man's diet. However, much of the vitamin E is apparently lost in processing. Whole wheat flour was found to contain about twice as much vitamin E as 80 per cent-extraction white flour.

The authors state that, in general, animal products are rather poor sources of vitamin E and that the concentration of this vitamin in animal products is probably directly proportional to the vitamin E content in the diet of the animal from which they came. It is also pointed out that the vitamin E content of animal products is adversely influenced by extensive processing and long storage.

The type of data obtained by these workers is of considerable importance even though at present it is not known whether vitamin E is a dietary essential for man. Vitamin E has been used in human patients under such diverse conditions as habitual abortion and heart disease. A critical review of the use of vitamin E in heart disease has recently appeared (*Nutrition Reviews* **7,** *118 (1949)*).

Protein-free Diet and Hypertension

The relation between diet and high blood pressure, in the mind of the layman and, at times in the mind of the physician, has always been intimate. There is strong evidence to indicate that structural abnormalities of the kidney play a major role in hypertension in man (*Nutrition Reviews* **2,** *188 (1944)*). It has been reported that a rise in blood pressure in rats occurs when they are fed a diet deficient in the heat-stable fraction of the vitamin B-complex (R. M. Calder, *J. Exp. Med.* **76,** *1 (1942)*). Restrictive diets in the treatment of high blood pressure in man have been in common usage for many years.

During the past few years interest in this subject has been stimulated by reports by W. Kempner (*North Carolina Med. J.* **6,** *61*

(*1945*)) that patients with hypertension maintained on a "rice diet" show improvement. Since this work a number of studies have appeared concerning the effect of nutrition on hypertension in man. Some success in lowering the blood pressure by simply restricting the sodium intake has been noted (A. Grollman *et al.*, *J. Am. Med. Assn.* **129,** *533* (*1945*)). Others (G. A. Perera and D. W. Blood, *J. Clin. Invest.* **26,** *1109* (*1947*)), using a rigid salt-restriction diet, reduced the blood pressure little; added salt produced only a slight rise. None of these effects was marked.

J. Brozek, C. B. Chapman, and A. Keys (*J. Am. Med. Assn.* **137,** *1569* (*1948*)) reported the effect of the famine in Leningrad on blood pressure. Following this famine there was a reduction in the incidence of hypertension and the symptoms related thereto, and there was a reduced incidence of myocardial infarction and angina pectoris. Following relief of the siege of Leningrad, when food became available, hypertension reappeared.

J. Kohári-Kucharik (*Am. J. Med. Sci.* **219,** *27* (*1950*)) had an opportunity to study a large population for many months following the siege of Budapest in the recent war. Because he was in the department of medicine of the university he was able to observe the effect of an imposed protein-free diet on hypertensive blood pressure in man. He refers to the work of S. Strouse and S. R. Kelman (*Tr. Assn. Am. Physicians* **37,** *166* (*1922*)) who reported a difference in blood pressure of monks of the Benedictine and Franciscan orders. There was a difference in blood pressure of some 30 to 40 mm. of mercury, on the average, and it was attributed to the fact that the orders with the lower blood pressures were vegetarian. The author reported that there was a complete protein famine following the siege of Budapest in 1945. This allowed him to make observations on the value of an animal protein-free diet on hypertension, a situation which could not readily be produced under experimental conditions. The observations were carried out on the outpatients of their medical department. Most of the patients had been under treatment for two to five years and had attended the outpatient clinic regularly every fourteen days. Only 39 of the original 150 patients were "available" for study. The author seemed certain that during this period the entire population of Budapest was deprived of animal protein for eight to ten months. In the table in the text the author gives the age, sex, and blood pressure readings at the end of 1944 and the readings in October and November, 1945. The average rise or fall of blood pressure in millimeters of mercury is also given in each case. The average change in the entire group was +4.6 mm. of mercury. These 39 patients during the ten months on an animal protein-free diet did not show any consistent change in blood pressure after this long period of animal protein deprivation.

Obviously this study was done under circumstances that did not permit the detailed study that the author would have liked to carry out. At the same time it does record some data in unusual circumstances, and certainly under the conditions of the experiment the deprivation of protein had little or no effect on hypertension in man.

Recent Nutrition Books

The Biology of Human Starvation. Ancel Keys, Josef Brozek, Austin Henschel, Olaf Mickelsen, and Henry L. Taylor. University of Minnesota Press, Minneapolis, Minnesota. 1950. (2 volumes). Price: $24.00.

Food for Fifty. Sina F. Fowler and Bessie B. West (3rd Ed.) John Wiley & Sons, Inc., New York 16, N. Y. 1950. Pp. 444. Price: $4.50.

Activities—in Nutrition Education. A Unit for High School Biology Classes. Elizabeth A. Lockwood. Copies may be ordered from The Nutrition Foundation, Inc., Chrysler Building, New York 17, N. Y. 1950. Pp. 92. Price: $1.00.